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Repair Process of DNA Double Strand Breaks Induced by X-ray Bystander Effect

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1 Introduction

Recent evidence suggests that damage signals may be transmitted from irradiated to non-irradiated cells in a population, leading to the induction of genetic changes that include gene mutations in bystander cells that received no radiation exposure. This phenomenon, the radiation-induced bystander effect, has been observed in mainly fibroblast and epithelial cells by assay for various biological endpoints [1-3]. In the previous study, we investigated the repair kinetics of DNA double strand breaks (DSB) in non-irradiated primary normal human fibroblasts (MRC-5) co-cultured with 20 mGy-irradiated MRC-5. After 48 h of co-culture, 81% of the initial numbers of DSB remained in non-irradiated MRC-5 [4]. In addition, when MRC-5 were irradiated with 1000 mGy after co-cultivate with 20 mGy-irradiated MRC-5, we found that the numbers of DSB significantly decreased compared with 1000 mGy-irradiated MRC-5 which were not experienced co-cultivate with 20 mGy-irradiated MRC-5 (under preparing submission). From these previous findings, we hypothesized that DSB resulting from the radiation-induced bystander effects might not be repaired, and unrepaired DSB by radiation-induced bystander effect might contribute to induction of radioadaptive response. In the present study, as the first trial to prove this hypothesis, we investigated a dose-response of DSB in both X-irradiated MRC-5 and bystander MRC-5 using by X-ray microbeam.

2 Materials and Methods

Cell culture. Primary normal human fibroblasts from the lung, MRC-5 (European Collection of Cell Cultures), were grown on a sterilized cover glass in MEM supplemented with 10% fetal bovine serum and penicillin-streptomycin at 37°C in a humidified incubator with 5% CO_2. All experiments were performed using non-dividing confluent cell cultures, the confluent state was kept for at least 24 h before experiments, in order to eliminate disparate cell-cycle phase radio-sensitivities.

X-ray microbeam irradiation. X-ray rectangular microbeam was delivered as a size of 2 mm x 1.3 mm with 5.35 keV at BL-27B. Dose rate was 20 R/s.

Dose-response curve of DSBs in both bystander MRC-5 and X-irradiated MRC-5. Cover glass with confluent cells was put on a Mylar sheet. After irradiation with doses of 0.5 Gy, 1 Gy, 5 Gy and 10 Gy, cells were incubated for 10 min at 37°C under 5% CO_2. Subsequently DSB was detected by 53 binding protein 1 (53BP1) immunofluorescent staining.

3 Results and Discussion

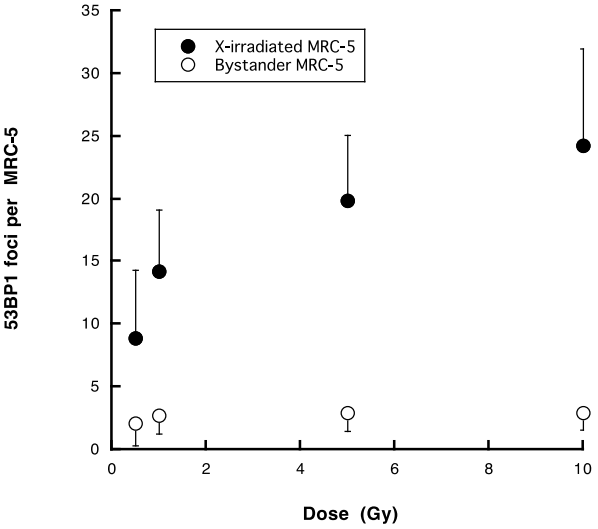

Fig. Dose-response curve of DSB in X-irradiated MRC-5 and Bystander MRC-5

The numbers of DSB in X-irradiated MRC-5 and in bystander MRC-5 were determined by assessing the number of 53BP1 foci. The dose-response relationship for the number of DSBs in X-irradiated MRC-5 was supralinear (Fig ●). However, the number of DSBs in bystander MRC-5 was non-linear dose response curve (Fig ○). In the next step, to verify whether the signals transmitted by X-irradiated MRC-5 are able to cause unrepaired DSB in bystander MRC-5, we will investigate the loss of 53BP1 foci in bystander MRC-5. Furthermore, we will investigate whether the radioadaptive response is observed in bystander MRC-5 with unrepaired DSB.

References

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